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The Effects of Gonadotropin Releasing Hormone and Milk Production on Pregnancy Rates in Repeat Breeder Dairy Cows

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THE EFFECTS OF GONADOTROPIN RELEASING HORMONE

AND MILK PRODUCTION ON PREGNANCY RATES

IN REPEAT BREEDER DAIRY COWS

by

Laura Maureen Mitchell

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Laura Maureen Mitchell

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ABSTRACT

The Effects of Gonadotropin Releasing Hormone
and Milk Production on Pregnancy Rates
in Repeat Breeder Dairy Cows

by

Laura M. Mitchell, Master of Science
Utah State University, 1988

Major Professor: Dr. Robert C. Lamb
Department: Animal, Dairy and Veterinary Sciences

One hundred sixteen third- and fourth-service lactating dairy cows from five cooperating herds were used to compare the pregnancy rate of cows given Gonadotropin Releasing Hormone five minutes following insemination with pregnancy rate of control cows. In addition, milk production level, age at service and days in milk at time of service were used to compare pregnancy rates. Milk production level was expressed as relative value (percent of herd mature equivalent production). There were no differences between treated and untreated control cows in pregnancy rate. In addition, there were no differences between service number (3 or 4), relative value group, age at service, days in milk at time of service, treatment by service, treatment by relative value, service by relative value, age squared and days in milk squared in pregnancy rate.

(50 pages)

STATEMENT OF THE PROBLEM

Introduction

High reproductive efficiency is important for maintaining a profitable dairy herd. Low conception rates of dairy cattle result in economic losses to the dairy producer by reducing reproductive efficiency and consequently reducing overall milk production. Poor reproductive performance ranks second only to milk production as a reason for culling (3).

One of the most costly reproductive problems in domestic animals is multiple services per conception. While there are many factors that contribute to poor conception rates, few can be more bothersome to the producer than apparently normal cows that fail to conceive after multiple services. "Repeat breeding" has been defined as failure to conceive from three or more regularly spaced services in the absence of detectable abnormalities (43). Studies have shown the incidence of repeat breeding to be between 5.0 and 15.1% (43).

Among the most significant recent advances in the field of bovine reproduction is the commercial availability of Gonadotropin-Releasing Hormone (GnRH). Recommended use is to inject GnRH at the time of breeding, between the endogenous surge of LH and ovulation (8). Administration of GnRH causes dose-related increases in serum concentrations of FSH and LH in cattle (8, 49). The LH surge terminates growth and maturation of granulosa cells and aids in their differentiation to luteinized cells. It is thought that GnRH induces active luteinization of granulosa cells, ensuring adequate production of progesterone to maintain pregnancy. However, administration of

GnRH near the time of ovulation has yielded variable results in relation to improving pregnancy rates.

Even with these variable results, GnRH is currently being recommended as a treatment for third- and fourth-service cows. Routinely using the hormone raises a valid question of cost, since administering GnRH to certain cows may not be cost effective.

High milk yield and lowered reproductive performance are thought by many dairy producers to be closely linked. Factors such as increased days open (31) and a longer calving interval (41) have been observed for higher producing cows. However, very few studies have reported the correlation between milk production and pregnancy rate. A Swedish study (23) indicated a negative correlation between milk production and reproductive performance, with repeat breeder cows producing 86.4 kilograms more 4 percent fat-corrected milk than control cows. The difference was statistically significant.

Problem Statement

GnRH is currently being recommended as a treatment to increase pregnancy rate in third- and fourth-service cows, even though field research indicates highly variable results. Although high milk production is thought by many producers to adversely affect fertility, few studies have been conducted to establish this relationship.

Purpose and Objectives

This study was designed to determine the relationship of the following factors in repeat breeder cows:

1. The effects of injecting GnRH at the time of breeding on pregnancy rate.

2. The effects of level of milk production on pregnancy rate.
3. Variation in response to GnRH in cows of different milk production levels.

Hypotheses

1. GnRH treated cows will exhibit a higher pregnancy rate than control cows.
2. High levels of milk production will adversely affect pregnancy rates.
3. Cows of different milk production levels will respond differently to GnRH administration.

Definition of Terms

1. Repeat breeding: Failure to conceive from three or more regularly spaced services in the absence of detectable abnormalities.
2. Reproductive efficiency: Includes interval to first estrus, interval to first breeding, days open, first service conception rate, services per conception, calving interval and other measures of reproduction.
3. Services per conception: Number of services required for a cow to conceive.
4. Conception rate: Number of cows which conceived per number of cows bred at a specific time x 100.
5. Pregnancy rate: Number of cows diagnosed pregnant per number of cows bred at a specific time x 100.
6. Reproductive performance: A general term used to include any or all aspects of reproduction.

REVIEW OF LITERATURE

Introduction

The overall objective of today's dairyman is to make a profit in his business. Maintaining a profitable dairy herd is contingent upon high reproductive performance. Low conception rates of dairy cattle result in economic losses to the dairy producer by reducing reproductive efficiency and thereby reducing milk yield.

Reduced reproductive performance ranks second only to low milk production in terms of reasons for culling (3). The incidence of culling due to poor reproductive performance has increased dramatically in recent years. Based upon Dairy Herd Improvement (DHI) data, 8-10 percent of the nation's dairy herd is culled annually because of reduced reproductive performance (23).

Ideally, calving intervals should be 12-13 months (34), thus necessitating cows to be pregnant by 85-115 days postpartum (44). Beyond 90 days postpartum a nonpregnant cow costs the dairyman \$1.50 to \$2.00 per day for excess feed and labor and reduced milk production (23). The cumulative loss due to infertility includes both culling losses and losses due to excessively long calving intervals. Conservatively, infertility costs United States' dairy producers \$960 million annually (23).

Many factors contribute to poor conception rates when using artificial insemination (AI) including improper handling of semen, poor AI technique, misdiagnosed estrus, and improper timing of inseminations (52). However, few problems can be more frustrating to the producer than cows that continue to return to estrus and fail to

conceive after repeated inseminations. "Repeat breeding" has been defined as failure to conceive from three or more regularly spaced services in the absence of detectable abnormalities (43). Economically, repeat breeding is one of the most important reproductive problems, as the calving interval is extended by at least three estrous cycles or two months (43). Studies have shown the incidence of repeat breeding to be between 5.0 and 15.1% (43).

Causes of Repeat Breeding

Etiologically and pathologically, failures in conception are complex (43). Pathologically, conception failure is the result of either fertilization failure or early embryonic mortality (43). Results from several investigations, including one by Ayalon (4), indicate that early embryonic mortality rather than fertilization failure is the major cause of infertility in both repeat breeders and control females. In cattle, early embryonic death must occur before the sixteenth or seventeenth day after service to allow for regular recurrence of estrus. Ayalon (4) found that fertility losses in dairy cattle, expressed as number of normal embryos per embryo found, are not significantly different between normal and repeat-breeder cows until day 6 or 7 after insemination. At 4 to 5 days after insemination 88% of normal cows and 80% of repeat-breeders have normal embryos. However, by day 6 or 7, 83% of normal cows and only 42% of repeat breeder cows have normal embryos. The critical period appears to be soon after the embryo enters the uterus, 6 to 7 days after service, when the morula is developing into a blastocyst. These

results furnish clear evidence that embryonic mortality in repeat-breeder cows occurs quite early.

Early embryonic death may be caused by inadequacy of the uterine environment (43). Ayalon (4) compared the levels of carbohydrates, total protein and several ions in uterine flushings from cows with normal and degenerated embryos 6-8 days postinsemination. A significant difference in ion concentration was found, particularly on day 7 after estrus. Cows with abnormal ova have significantly higher concentrations of potassium, zinc, phosphorus and calcium. Concentration of calcium ions is nearly twelve times greater in cows with abnormal embryos. Total protein levels are consistently higher in normal cows. Both protein levels and ion concentrations may be related to the differences in quantity of cytosol and progesterone receptors in the endometrial cells.

Fertilization failure has been charged with causing a lowered pregnancy rate. Graden et al. (22) recovered 104 ova from 150 cows and reported that fertilization failure occurs in 20% but possibly in as many as 60% of repeat breeders. Abnormalities causing fertilization failure measured three days postinsemination were as follows: ovulation failure, 8.7% of the cows bred; oviduct obstructions, 6.7%; abnormal ova, 3.3%; ovarian adhesions 2.0%; endometritis, 3.3%; and lost ova, 17.3%. In 24.7% of the cows there was no apparent explanation for fertilization failure. Ayalon (4) found that fertilization failure or embryo non-survival to 35 days postinsemination are two to four times higher for repeat breeders than for normal cattle.

In the female, physiological events leading to conception are numerous. The neuroendocrine system is primarily responsible for the proper sequence and synchrony of a very complex system and series of events. A single asynchrony or multiple factors may result in a failure to conceive. Occasionally, females experience improper timing of the complex events that lead to estrus, ovulation and ultimately pregnancy. In addition, there are other disorders in the female causing conception failure. Among them are: defective oogenesis, degeneration of ovulated normal eggs, disorders of ovulation, inflammation of the ovary, hydrosalpinx, perisalpingitis, salpingitis, functional disorders of the oviducts, metritis, endometritis, inadequate endocrine support to the uterus, parametritis, white heifer disease, disorders of the cervix, vagina and vulva, and various diseases (43).

In a study by Maurer and Echternkamp (37), repeat-breeder and control cows were slaughtered 2 to 51 days postmating. They found the causes of repeat breeding in cattle could be classified as: reproductive tract aberrations, 10.9%; anovulation, 3.6%; chromosomal abnormalities, 14.3%; nonrecovery of either an oocyte or embryo, 34.7%; and endocrine dysfunction and other causes, 36.5%.

There have been studies that correlate poor nutrition with a reduction in normal fertilized ova and reduced levels of plasma progesterone. In dairy cows, level of nutrition affects plasma progesterone concentrations in cows failing to conceive at first insemination (18). Research in Israel (20) has shown a significant correlation between levels of roughage, digestible protein and energy and the incidence of repeat breeding in high-producing dairy herds.

Proper levels of these three constituents in the ration are essential to obtaining optimum fertility in high producing dairy cattle. Jordan et al. (26) demonstrated that the crude protein content in the diet alters uterine secretions of urea, magnesium, potassium, phosphorus and zinc. However, in other studies rations were shown to have remarkably little effect on ovarian function and services per conception (14, 15).

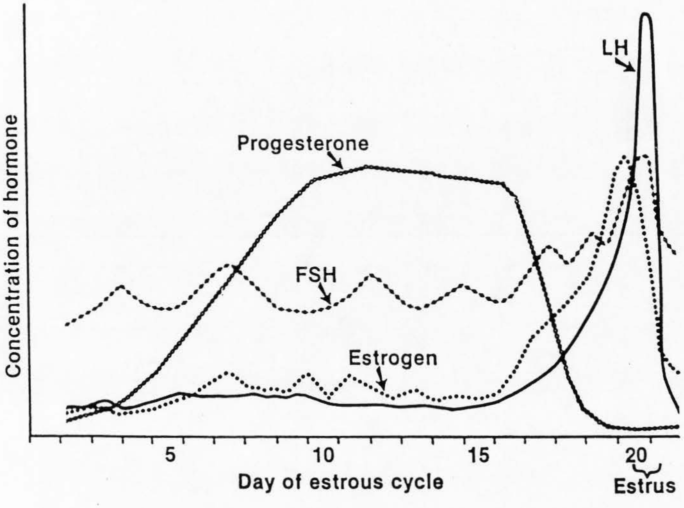
Endocrinology of the Estrous Cycle

Developments in endocrinology have lead to an increase in the knowledge and understanding of the physiologic mechanisms which control reproductive processes in animals. One of the most significant developments in the field of bovine reproduction in the past ten years has been the commercial availability of gonadotropin-releasing hormone (GnRH) and its analogs (8). GnRH is a decapeptide of hypothalamic origin which stimulates the release of the pituitary gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). It is also referred to as luteinizing hormone/follicle-stimulating hormone-releasing factor (LH/FSH-RF), luteinizing hormone-releasing factor (LH-RF) or luteinizing hormone-releasing hormone (LH-RH).

During normal mammalian estrous cycles, there is a delicate balance and feedback between the hypothalamus, anterior pituitary and target tissues. Generally, neurohormones from the hypothalamus initiate release of anterior pituitary hormones which then stimulate or depress biochemical reactions in the reproductive organs. Hormones

produced within the reproductive organs in turn may stimulate or depress synthesis of neuro and anterior pituitary hormones (1).

The hypothalamus initiates release of LH and FSH by secreting releasing and/or inhibiting neurohormones from the basal hypothalamus, which pass to the pituitary via the portal vessels. The anterior pituitary releases FSH and LH. FSH is carried via the blood to the ovary where it initiates follicular growth. As the follicle enlarges estrogen secretion is enhanced (53). The most obvious effect of estrogen is that it brings about estrus. LH causes ovulation and stimulates progesterone secretion which regulates luteinization of



Source: H. D. Hafs and L. J. Boyd. 1981.

Figure 1. Hormonal concentrations during the estrous cycle.

mature follicles. The pre-ovulatory LH surge induces proteolytic enzyme synthesis which weakens the wall of the graffian follicle, causing it to rupture. Luteinization of the theca interna and granulosa layer begins immediately. The corpus luteum forms, and 4 to 5 days after ovulation progesterone production begins (1). Progesterone is primarily responsible for the maintenance of pregnancy (23). Figure 1, reprinted with permission (Appendix A and B), shows the concentration of hormones in the blood of a normal bovine with a 21-day estrous cycle (23).

Why GnRH May Improve Fertility

Injection of GnRH at the time of breeding occurs between the endogenous surge of LH and ovulation. Administration of GnRH causes dose-related increases in serum concentrations of FSH and LH in cattle (8, 49). Additional endogenous LH is released approximately 2-3 hours after administration of GnRH (17, 30).

Kaltenbach et al. (27) observed a rapid surge in LH commencing within 15 minutes after GnRH injection. Mean duration of LH surge is approximately 7 hours with the peak occurring at 2 hours. Serum levels of FSH also increase, approximately threefold, after GnRH administration. The increase did not begin until 45 minutes post-injection; however, the peak levels of FSH coincide in time with the highest levels obtained for LH.

The same study (27) also reported that the quantity of LH and FSH released is affected by the endogenous amount of steroid hormones. A relatively small response to GnRH given during the luteal phase of the estrous cycle indicates that high levels of progesterone may be

inhibitory. Conversely, increases in serum LH are obtained if GnRH is given near estrus when levels of estrogen are high.

The anterior pituitary's responsiveness to GnRH is enhanced by the stimulatory action of estrogen and the self-priming action of GnRH (33). Therefore, it is possible that GnRH induces active luteinization of granulosa cells. This ensures adequate production of progesterone to maintain pregnancy when successful fertilization occurs.

The concept of delayed ovulation implies asynchrony in the time relationship between an LH surge and ovulation or an insufficient LH surge to stimulate the series of events that culminate in ovulation (33). The LH surge terminates growth and maturation of granulosa cells and aids in their differentiation to luteinized granulosa cells whose progesterone synthesis will support the stages of pregnancy (24). Low progesterone concentrations during the luteal phase have been reported for repeat breeder cows (16).

McLeod and Haresign (40) synchronized ewes with a prostaglandin analog and then treated them with GnRH or saline at two-hour intervals from day 14 of the subsequent cycle until 24 hours after the onset of estrus. Treatment with GnRH induced episodic LH release which continued until the onset of a preovulatory LH surge. Mean plasma LH concentrations over the period were significantly greater for ewes treated with 250 ng GnRH than for the control group.

Lee et al. (32) found that 24 cows given GnRH at insemination had higher LH concentrations in blood serum following treatment than cows given saline, 13.2 versus 3.0 ng/ml. Cows treated with GnRH that conceived had higher progesterone levels than all other cows. These

observations agree with Folman et al. (18) and Fonseca et al. (19). The higher progesterone produced by the GnRH-pregnant group was probably due to the surge of LH following administration of GnRH, which recruited more granulosa cells to become luteal cells for progesterone production, or enhancement of progesterone production by existing cells. This may explain the higher fertility in cows receiving GnRH (33, 44, 52).

These results are in near agreement with Fernandes et al. (17) who reported that LH increased within five minutes after GnRH injection. Peak LH concentrations occurred at about 120 minutes after GnRH treatment, and plasma concentrations returned to basal levels by 5 to 6 hours. Maurer and Rippel (38) reported a similar LH peak. Fernandes et al. (17) also discussed the inhibitory effect of progesterone and the correlation between estrogen and LH release.

Boice (7) discussed the importance of progesterone concentrations in supporting pregnancy and reported that 14% of cows in the early postpartum period did not produce sufficient progesterone to support pregnancy had it occurred. Progesterone concentrations during the estrous cycle prior to breeding may be an important indicator of the cow's subsequent fertility (19). Remsen et al. (48) found a significant relationship between progesterone concentration and subsequent pregnancy in recipient heifers. Bulman and Lamming (11) agreed that repeat-breeder cows have a lower progesterone concentration in the luteal phase than normal cows.

Erb et al. (16) reported that pregnancy failure may be associated with subnormal concentrations of progesterone prior to LH increase and

chronically low plasma progesterone during certain periods after ovulation. Maurer and Echternkamp (36) found higher embryo survival in cows with higher preovulatory surges of LH and earlier post ovulatory rises of luteal progesterone.

GnRH was given to repeat-breeder heifers to enhance and/or hasten corpus luteum formation and progesterone secretion in a different study by Maurer and Echternkamp (37). Although treatment did not increase pregnancy rate, progesterone concentrations increased in repeat-breeder heifers. Using frequent blood sampling in normal cows and heifers, they did find that females with normal developing embryos had higher progesterone concentrations on days 3 and 6 than females with abnormal embryos.

Thibier et al. (54) enhanced the rate of recovery and quality of the embryos in 101 repeat-breeding cows by using a GnRH-analog injection. They were able to recover 65% of the embryos from treated animals versus 32% in controls. Similarly, the treated group had 91% fertilized ova (in the proper stage of development) of those recovered, compared with 57% in untreated controls. This leads to speculation of better-synchronized expulsion of the oocyte with the oviduct capture, better quality of the gametes favoring a lower rate of embryonic mortality, and a better progesterone-secreting corpus luteum.

Previous Studies With GnRH

According to Bosu (8), systemic administration of GnRH using various routes, including intravenous, intramuscular, subcutaneous,

intravaginal and intrauterine, yield similar results in cattle. However, the most common method of administration is intramuscular.

GnRH has been used in bovine reproduction for treating cystic ovaries, maximizing the ovulatory rate with artificial insemination, treating ovulatory abnormalities, and managing postpartum activity. Reports indicate that GnRH may increase fertility in early postpartum cows by decreasing the interval from calving to conception, increasing first service conception and enhancing overall conception (4, 6, 13, 21, 47, 56). In addition, GnRH lessens the frequency (9, 29) and has been used effectively as a treatment for ovarian cysts (12, 43, 46).

The success of artificial insemination is dependent upon the proper timing of insemination relative to ovulation. Problems associated with estrus detection and variations in estrus duration may result in breeding at the wrong time of estrus. Highest conception rates can be expected if inseminations occur 6 to 24 hours before ovulation (52). Other work (50) indicates insemination should take place 7 to 18 hours before ovulation for highest conception rates. This can be achieved by administering GnRH during estrus to ensure the proper timing of ovulation.

Ovulation failure is one of the causes of fertilization failure in cattle. The incidence of ovulation failure has been reported to be 2-19% (45). It has been recommended that cows which do not ovulate within 24 hours after insemination be reinseminated and treated with luteotropic substances at the next estrus (43). Prophylactic treatment may be given at the time of insemination in cows predicted to fail to ovulate (45).

Nakao et al. (45) administered GnRH to 72 cows in which ovulation failure was predicted by rectal palpation and by repeat breeding. In those treated, 86.8% ovulated and 51.4% conceived. The ovulation rates following treatments were not significantly affected by the age of cow, number of inseminations or interval from parturition to treatment.

In a different study by Nakao et al. (44), 605 first-service cows were administered GnRH intramuscularly. A 7.5% improvement in pregnancy rates was reported ($P<.05$). GnRH injected at insemination was effective, especially in cows at the first or third lactations, cows at 101 days postpartum or later, cows with daily milk yield of 26 to 30 kg, and in cows from the area where a regional average fertility was low. They theorized that cows having a milk yield of 31 kg or higher may fail to conceive due to other causes than the delayed ovulation.

Research in Iran by Schels and Mostafawi (50) showed a similar first-service pregnancy rate increase of 9.2% in 64 GnRH-treated cows ($P<.18$) Services per conception were reduced from 1.49 in controls to 1.39 in GnRH-treated cows. Total pregnancy rate of 81.65% was 8.3% higher than in the controls ($P<.20$) They concluded that GnRH stimulated the release of LH, triggering ovulation, enabling contact between live ovum and sperm cells and allowing fertilization to take place.

Moller and Fielden (42) observed a 9.3% higher first-mating pregnancy rate in 292 GnRH-analog treated cows than in the control group. In addition, cows inseminated within 60 days of calving had a lower pregnancy rate than other cows. While both treatment and

postpartum interval had significant effects on pregnancy rate, there was no evidence of any interaction between them.

Lee et al. (33) worked with 346 repeat-breeder cows distributed across 26 dairy herds in Wisconsin. The cows were given either GnRH or saline solution. Repeat-breeder cows given GnRH instead of saline solution at the time of breeding had significantly higher conception rates, 73% versus 48%. There were no significant differences in average age, average milk production, days to first observed estrus, and days to first breeding between treated cows and controls.

In a Kansas State University study (52) involving 328 cows there was no effect of GnRH treatment at insemination and no effect of time of insemination on first-service conception rates. However, on second and third services, GnRH-treated cows tended to have higher conception rates than saline-treated cows. When repeat services were combined conception was improved 10 percentage points.

These results are in agreement with Schmidt et al. (51) who reported similar first-service conception rates among GnRH and saline-treated groups. However, conception rates at second (40 versus 57%) and third (55 versus 78%) services were higher in the GnRH than saline-treated cows.

Maurice et al. (39) reported that 46% of the cows receiving saline conceived at third service, compared with 71% of cows injected with GnRH. No differences in age and milk production were observed.

Brown (10) reported on two studies. In one, involving 185 cows, 73% of cows given GnRH at third service conceived while 48% of untreated repeat breeders conceived. In the other, involving 115

repeat breeders at second- or third-AI service, 63% of GnRH cows versus 55% of untreated repeat breeders conceived.

In a somewhat contradictory study, Kazmer et al. (28) found that pregnancy rates did not differ following GnRH treatment after synchronization of 55 heifers with prostaglandin. These results concur with a study cited by Thatcher and Chenault (53) in which GnRH administration following prostaglandin treatment depressed conception rates among beef cattle.

Anderson and Malmo (2) studied a total of 3502 first services, 1242 second services and 361 third services in 25 Australian herds. Of the 25 herds, the effect of treatment with GnRH at first service was: 10 herds showed a change (6 an increase and 4 a decrease) in pregnancy rate of less than 5%, 10 herds had an increase of 5% or more, and 5 herds had a decrease of 5% or more. Overall there was a significant increase in pregnancy rate from 54.1% to 58.8% associated with giving GnRH at first service. Treatment with GnRH at second service increased pregnancy rate from 56.6% to 58.1%. An overall decrease from 53.0% to 44.1% with giving GnRH at third service was observed. There was no interaction between treatment and interval from calving to service or herd.

Administration of GnRH to cows 1-3 days postinsemination resulted in a pregnancy rate of 54.5%, compared to 71.0% in untreated controls, in a study by MacMillan et al. (35). In the same study, administration from 7-10 days postinsemination resulted in a 13.2% increase in pregnancy. These results indicate that the treatment may have prolonged the lifespan of the corpus luteum, thereby increasing

the probability of maternal recognition of the presence of a developing embryo.

Studies have attempted to determine if GnRH administered near the time of insemination increases pregnancy rate. Results have yielded variable results. Furthermore, the mechanism under which GnRH may enhance pregnancy rate is not fully understood.

Relationship of Milk Yield With Fertility

A Swedish study (25) shows that the percentage of repeat breeders rises progressively as herd size increases. The highest incidence is in cows that calve between September and February. This work also indicated a correlation between milk yield and fertility. Repeat-breeder cows produced a highly significant 86.4 kilograms of 4.0 fat-corrected milk (FCM) more than control cows. The difference was not significant in primiparous cows. However, in cows which had at least one previous lactation, the repeat breeders produced an average of 78.6 kilograms of 4% FCM more than controls.

A direct relationship between amount of milk produced in the first lactation and a longer calving interval was observed by Miller et al. (41). Milk production was measured as deviation from herd mates and showed that the highest producers within the herd had longer calving intervals than their contemporaries that produced less milk.

Kragelund et al. (31) found the phenotypic relationship between days open and milk production in the succeeding lactation was positive. Genetic correlation between milk yield and days open were .62 to .72, indicating a close connection between breeding values of milk production and days open.

More days open are associated with an extended late lactation period where daily production is low. Whereas, fewer days open are similarly identified with a shorter period of low daily production (34).

Louca and Legates (34) found that maximum production is attained when cows have the least number of days open. A calving interval of 13 months for primiparous cows and 12 months for multiparous cows is suggested as an optimum length for attaining maximum production. These results concur with Bar-Anan and Soller (5).

Wood and Frappell (55) observed that cows with longer calving intervals tend to produce more milk than those with shorter ones. In studying 1475 lactation records of 808 daughters of eight bulls, they noted cattle requiring more than one insemination to become pregnant produced 173 kilograms more milk in 305 days than those requiring only one insemination.

Fonseca et al. (19) reported that cows whose lactation yields range from 0 to 1000 kilograms below herdmates have the fewest days to first insemination whereas cows that produce higher have progressively longer intervals to first insemination. Each 100 kilogram increase in deviation from herdmates result in a .6 to 2.0 day increase of days open. Furthermore, cows that produce between 1400 and 2600 kilograms during 70 days postpartum have similar conception rates. Conception rate increase for cows that have less than 1400 kilograms and decreases for cows that produce more than 2600 kilograms.

MATERIALS AND METHODS

Cooperator Selection

This study was undertaken utilizing dairy cows from five cooperating Utah dairymen. Selection of the cooperating dairy herds was based on information provided by dairy extension specialists and DHI records from Utah and Idaho dairy farms. Following consideration of the data available, ten prospective cooperators were contacted and the proposed study was outlined. Of the ten, five were selected, all located in Utah. The final selection was based on the following criteria: willingness to participate in the study, accurate record keeping, willingness to allow access to DHI and herd health records, exclusive use of artificial insemination (AI) on treatment cows, participation in a regular herd health program, and milking at least 200 head of dairy cows.

Cooperators were expected to artificially inseminate the cow, administer the treatment, record appropriate data and obtain pregnancy diagnosis. Additional changes in management were not required. The duration of the study was from June 12, 1986 to January 27, 1987.

Criteria for Animal Selection

Approximately one month prior to inclusion in the study, all cows were examined by a veterinarian who determined, through rectal palpation, that cows were reproductively sound and cycling normally. Regular access to herd health records was mandatory. Animals used for the study were non-pregnant after two services. Only third-service cows were used to start the study. If the cows returned into estrus after third service, they were again treated and designated as fourth-

service cows. Cows were primiparous and multiparous, with no age limitations. All cows included in the study were Holsteins.

There was a total of 116 third- and fourth-service observations, with 90 being third service and 26 being fourth service. A total of 90 cows were used in the study, with 44 being treated and 46 being controls.

Treatment and Procedure

There were two treatments: A) control (2 ml saline solution); B) treated (2 ml GnRH).

Third service cows were assigned alternately, as they displayed estrus, to either treatment or control and remained in that group for the duration of the study. This distributed the possible effects of such variable factors as season, technician, service sire, feeding, and management equally over the two groups.

GnRH and saline solution were provided to the dairymen in similar 2 ml vials ensuring ease of single-dosage administration. GnRH (Cystorelin®) was provided by Ceva Laboratories, Overland Park, Kansas. Cystorelin, a decapeptide, is a sterile solution containing 50 micrograms or gonadorelin diacetate tetrahydrate per milliliter. The recommended intramuscular dosage is 100 mcg (2 ml) per treatment. Mention of the trade name does not imply an endorsement or recommendation by Utah State University over similar companies or products not mentioned.

Commercial labels were removed from the bottles and the bottles were precoded A or B to prevent the dairymen from knowing the contents. The A bottles were control and B were the treatment.

Alcohol pads, disposable syringes, needles, instructions (Appendix C), and a record of drug usage (Appendix D) were also provided. Cows were artificially inseminated approximately 12 hours after observation of standing heat. When possible, all cows within each herd were inseminated by the same technician, usually the farm manager or herdsperson. Treatment with GnRH or saline solution was administered within five minutes after artificial insemination. When possible, distribution of service sire within the two groups was similar. At the time of injection, individual cows, identified by DHI control numbers, were recorded as having been given solution A or solution B. In addition, the date, technician name, service number, and service sire were recorded on supplied forms (Appendix E).

Pregnancy status was determined by return to estrus (open cows) or by rectal palpation 30-75 days after insemination. Cows that returned to estrus within 50 days after third insemination were retreated with the same treatment at fourth service. This was to ensure there were no crossover effects. A total of 116 injections were given, 90 being at third service and 26 at fourth service.

All herds were visited approximately once a month and data sheets were collected for statistical analysis.

Milk Production Groups

DHI records were obtained from cooperator herds for statistical evaluation (Appendix F).

Milk-production records were standardized to 3.5% Fat-Corrected-Milk (FCM) by using the following formula: $0.4318M + 16.2238F = 3.5\%$ FCM, where M = extrapolated 305-day milk production and F =

extrapolated 305-day butterfat production. To equalize age and length of lactation differences, all records were estimated on a 305-day, two-time milking, mature equivalent (ME) basis.

Relative value was used to assign cows into milk production groups as data were collected. The relative value used is expressed as "% of herd, ME" on DHI records and indicates the percent a cow is above or below other cows in the herd. Herd average is 100 percent. Relative value was obtained by the following formula: $R = (I/H) \times 100$, where R = relative value, I = extrapolated 305-day mature equivalent milk production for the individual cow, and H = monthly extrapolated 305-day mature equivalent milk production for the herd. Monthly recomputation was necessary to enable each succeeding computation to be a more accurate estimate of the completed 305-day mature equivalent lactation. In addition to providing relative value, DHI records were used to determine days in milk and age at service.

Prior to the initiation of the study, records were obtained from the DHI Computing Center in Provo, Utah, for seven randomly selected Utah dairy herds (Appendix F). Cows within each herd were arranged in descending order according to relative value and were divided into three groups with an equal number of cows in each group. After combining the data from all herds, the relative values for the three groups were: high 107 and above, medium 93-106, and low 92 and below.

Management of individual cows was not altered due to the assignment into milk-production groups.

Statistical Analysis

The effects of GnRH and milk production on pregnancy were

analyzed using least squares analysis. Third and fourth services were both included in the first analysis. Factors included in the model were treatment, service number, relative value group, treatment by service, treatment by relative-value group, service by relative-value group, age at service, age squared, days in milk at service, and days in milk squared. Herd effects were not included in the model, as there were only small numbers of cows within each treatment within each herd.

A second analysis of variance using a least squares model was then performed. Only third service animals were included. Factors included in the model were treatment, relative value group, treatment by relative value group, age, age squared, days in milk at service, and days in milk squared.

A stepwise regression using only third service cows was performed to determine the maximum R-square improvement attributable to each independent variable, with the dependent variable being pregnancy. The effects of the GnRH treatments on pregnancy rates were further analyzed by using chi-square comparisons of the percentage confirmed pregnant and the percentage remaining non-pregnant. As there were only small numbers of fourth services, third and fourth services were combined, with fourth service being treated as a separate observation. Similar analysis were completed only for third-service cows.

RESULTS AND DISCUSSION

This study was conducted with the cooperation of five Utah dairy farms. There were a total of 116 third- and fourth-service observations, with 90 being third service and 26 being fourth service. The results of the analysis of variance using third and fourth services are in Table 1.

Table 1. Analysis of variance using third and fourth services.

Source of Variation	DF	Sum of Squares	F Value
Model	13	3.104	0.97
Treatment (Trt)	1	.001	0.00
Service (Serv)	1	.081	0.33
Relative Value (RV)	2	.460	0.94
Trt X Serv	1	.025	0.10
Trt X RV	2	1.065	2.17
Serv X RV	2	.319	0.65
Age	1	.152	0.62
Age Squared	1	.121	0.49
Days in Milk	1	.990	4.03
Days in Milk Sq	1	.778	3.17
Error	102	25.034	.97
Total	115	28.138	

There were no differences ($P>.05$) between treated and untreated controls in pregnancy rate using 116 total third and fourth service observations. These results are in agreement with Kazmer et al. (28) and Thatcher and Chenault (53), but contrast those of Lee et al. (33), Maurice et al. (39), Nakao et al. (45) and Schmidt et al. (51), who found increased pregnancy rates following GnRH administration. Anderson and Malmo (2) found that GnRH administered at third service decreased pregnancy rate.

In addition, there were no differences (P>.05) between service number (3 or 4), relative value group, age at service, days in milk at time of service, treatment by service, treatment by relative value, service by relative value, age squared and days in milk squared in pregnancy rate.

Age in months at time of service ranged from 24 to 137 (2 years to 11 years, 5 months), with the mean being 55.30 months (4 years, 7 months) and the standard deviation being 22.49 months. Days in milk at the time of service were between 82 and 298. The mean was 144.48 days with a standard deviation of 42.91 days. There were no differences in pregnancy rate among different ages or days in milk at service. Other factors, including service sire, technician, and date of prior veterinary examination, were recorded but not analyzed because of limited information within each group. Between-herd comparisons were not performed in this model, as there were limited numbers of cows in each herd.

Using only the third-service cows (n = 90), relative value ranged from 70 to 153 percent of herdmates based on 3.5% Fat-Corrected Milk. The mean relative value was 100.23 with a standard deviation of 14.97. There were 30 (33.3%) high, 34 (37.8%) medium and 26 (28.9%) low relative-value cows using the grouping system from the preliminary study. Results of the analysis of variance using only third-service cows are in Table 2. There was no difference (P>.05) between treatment, relative value, treatment by relative value, age, age squared, days in milk or days in milk squared using only third service.

A stepwise regression using only third-service cows to determine the maximum R-square improvement attributable to each of the following variables: age, age squared, days in milk and days in milk squared failed to attribute significance to any variable for either treatment.

Table 2. Analysis of variance using only third service.

Source of Variation	DF	Sum of Squares	F Value
Model	9	2.641	1.21
Treatment (Trt)	1	.017	0.07
Relative Value (RV)	2	.389	0.80
Trt X RV	2	1.055	2.17
Age	1	.207	0.85
Age Squared	1	.291	1.19
Days in Milk	1	.744	3.06
Days in Milk Sq	1	.568	2.34
Error	80	19.459	
Total	89	22.100	

The effects of treatment on pregnancy rate, relative value on pregnancy rate, service on pregnancy rate and treatment on relative value, were further analyzed using chi-square comparisons. No differences were detected.

Of the 26 cows that were retreated at fourth service, 19 exhibited a normal estrous cycle (19-24 days). The remaining 7 were observed in estrus between 15 and 35 days after third insemination.

Table 3 shows the pregnancy rates of treatment and control cows within each herd. It was not possible to make a statistical analysis of these data due to limited numbers of cows within each herd. However, numerically herds 1, 2 and 3 exhibited a higher pregnancy rate following GnRH treatment than control cows, especially at third service. Pregnancy rates on GnRH-treated cows in herds 4 and 5 were

less than the untreated controls. These findings are in agreement with Anderson and Malmo (2), who studied Australian herds and found highly variable responses between herds. The lack of agreement between herds is difficult to explain but are most likely the result of differing management between herds. Perhaps there were unnoticed differences in accuracy of estrus detection, timing of insemination, insemination procedure or other managerial aspects. Herd 5 began breeding cows, first service, at approximately 30 days postpartum, while all other herds inseminated cows closer to the recommended 60 days postpartum. To a point, pregnancy rate in dairy cows is increased as the interval from parturition to first insemination is increased (47). In addition, herd 5 participated in a 6- to 8-week herd health program while all other herds were on a 2- to 4-week herd health schedule.

Such apparent herd differences may explain why previous studies have not been in agreement. Perhaps additional studies conducted over more herds would be able to pinpoint important managerial differences. This would enable individual herd managers to determine the economic benefit of using GnRH in their herd.

The largest limitation to field research with cooperating herds is the level of cooperation given by each herdsman. It should be noted that herds 1, 2 and 5 were extemely helpful throughout the duration of the study, and that even though the study was designed to obtain satisfactory numbers for statistical evaluation, total cooperation from every herd was necessary. Some herd managers did not treat all available cows and provide all the data they could have,

which prevented this study from being as informative as it might have been. Thus, in future studies of this type, more herds should be

Table 3. Herd comparisons of pregnancy rate.

	Control			GnRH		
Number of third service cows	Preg.*	Non-preg.	Preg. Rate %	Preg.*	Non-preg.	Preg. Rate %
Herd 1	4	7	36.36	5	6	83.33
Herd 2	5	4	55.55	7	3	70.00
Herd 3	2	4	33.33	4	1	80.00
Herd 4	2	2	50.00	2	3	40.00
Herd 5	7	9	43.75	5	8	38.46
Total	20	26	43.38	23	21	52.27
Number of fourth service cows						
Herd 1	2	4	33.33	2	2	50.00
Herd 2	1	2	33.33	1	2	33.33
Herd 3	0	0	-----	0	0	-----
Herd 4	0	0	-----	0	0	-----
Herd 5	1	2	33.33	2	5	28.57
Total	4	8	33.33	5	9	35.71
Number of third and fourth serv. cows combined						
Herd 1	6	11	35.29	7	8	46.66
Herd 2	6	6	50.00	8	5	61.54
Herd 3	2	4	33.33	4	1	80.00
Herd 4	2	2	50.00	2	3	40.00
Herd 5	8	11	42.10	7	13	35.00
Overall total	24	34	41.38	28	30	48.28

* Pregnant to that specific service.

involved in the original model to insure adequate numbers within each treatment group. In addition, more careful selection of herds, closer follow up and stricter supervision on the part of the researcher may be of benefit. Providing incentives to the cooperator for each cow included in the study might encourage cooperation.

CONCLUSION

Due to the limited number of animals tested in this study it was not possible to reach realistic conclusions regarding the relationship of GnRH, milk production and pregnancy rate. More research needs to be done to establish these relationships.

Selection of cooperator herds should be done with extreme care, and the project should be designed to ensure that adequate numbers of animals will be obtained to make statistically significant comparisons.

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APPENDIXES

Appendix A.
Request to Reprint Figure 1

November 5, 1987

Laura M. Mitchell
Route 38, Box 2090
Livingston, Montana 59047
Phone: 406-222-0150

Dear Sirs:

I am in the process of preparing my thesis in the Animal, Dairy and Veterinary Science Department at Utah State University. I hope to complete in the Fall of 1987.

I am requesting your permission to include the attached material as shown. I will include acknowledgements and/or appropriate citations to your work as shown and copyright and reprint rights information in a special appendix. The bibliographical citation will appear at the end of the manuscript as shown. Please advise me of any changes you require.

Please indicate your approval of this request by signing in the space provided, attaching any other form or instruction necessary to confirm permission. If you charge a reprint fee for use of your material, please indicate that as well. If you have any questions, please call me at the number above. I hope you will be able to reply immediately. If you are not the copyright holder, please forward my request to the appropriate person or institution.

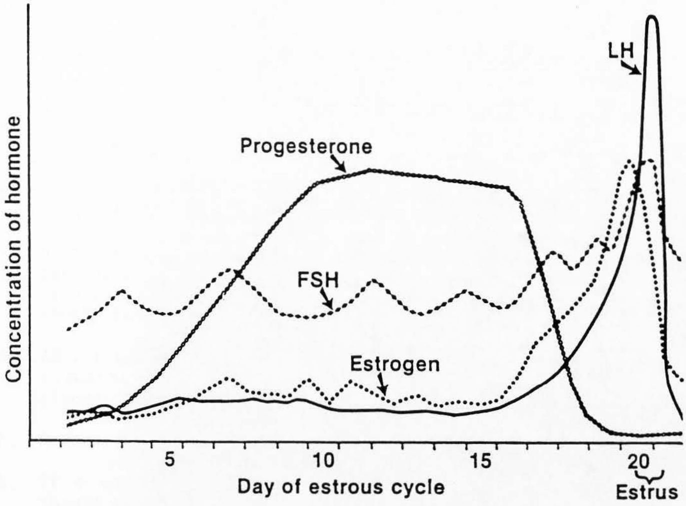
Thank you for your cooperation.

Most sincerely,

Laura M. Mitchell

Appendix B.
Release Form For Figure 1

I hereby give permission to Laura M. Mitchell to reprint the following material in her thesis.



Bibliographical entry:

23 Hafs, H. D., and L. J. Boyd. 1981. Dairy Cattle Fertility and Sterility. 3rd ed. W. D. Hoard and Sons Co., Fort Atkinson, WI.

(Fee)

(Signed)

Appendix C.
Instructions For Cooperators

1. Use only third and fourth service cows for the study. Begin with only third service cows. No fourth service cow shall be treated unless she was first given a treatment at third service. You may delete any cow from the study prior to initiation of the study. Twenty four cows from each herd will be needed.

2. Review herd health records to make sure that the cow has been checked by a veterinarian and is ready to breed.

3. Assign each third service cow alternately to a group (A or B) as she comes into heat.

4. Record cows Relative Value from her DHI records. Use the Relative Value figure from her most recent test.

5. AI the cow at the regular time using your regular practice. The same technician should AI all cows within the same herd, and if possible service sire distribution within the two groups (A or B) should be equal.

6. Administer the proper treatment intramuscularly immediately after insemination. Individual disposable syringes, needles and alcohol pads are provided.

7. Record the appropriate information on the data sheet provided.

8. If a cow returns into heat within 50 days after breeding she should be given the same treatment as before.

9. If a cow returns into heat more than 50 days after breeding she should not be reassigned to any group. It is the dairymans option for treatment. Please do not use the drugs supplied as part of this study to treat these cows.

10. If you have any questions please call me, Laura Mitchell, during the summer months at (406)-222-0150 or (406)-222-3208. Please ask for Laura and if I'm not available leave a message. I'll get back to you as soon as possible. After September 1, please call (801)-753-2427. My advisor is Dr. Robert Lamb, who can be reached at (801)-750-2159.

Appendix D.
Record of Drug Usage Provided to Cooperators

Farm Name ________________ Owner/Manager ________________

________ (date)	Delivered at beginning of study	____ (no.)	doses of B.
________ (date)	Delivered during the study	____ (no.)	doses of B.
________ (date)	Delivered during the study	____ (no.)	doses of B.
________ (date)	Delivered during the study	____ (no.)	doses of B.
	Total =	____	doses of B.
________ (dates)	Used as part of the study	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	________ (method of loss ex:dropped)	____ (no.)	doses of B.
________ (date)	Returned at the end of study	____ (no.)	doses of B.

____
(Signature)

Appendix E.
GnRH Data Report Form

Farm Name ____________________ Owner/Manager ________________________

DHI ID Number _______________ Veterinarian ________________________

DHI Cow#	Treat-ment	Date	Service Sire	Service Number	Prior Vet Check	Preg Check

Appendix F.
Obtaining Relative Value Groups

Herds used in the preliminary study to establish relative value levels for grouping of study cows.

Herd 87-03-0236

Relative Value	Group 1:	108-134	118
	Group 2:	94-107	100
	Group 3:	46- 93	81

Herd 87-03-0310

Relative Value	Group 1:	106-136	115
	Group 2:	93-105	100
	Group 3:	75- 92	83

Herd 87-03-0341

Relative Value	Group 1:	110-159	121
	Group 2:	96-109	103
	Group 3:	45- 95	82

Herd 87-21-0215

Relative Value	Group 1:	108-153	118
	Group 2:	93-107	100
	Group 3:	55- 92	81

Herd 87-25-0597

Relative Value	Group 1:	107-161	121
	Group 2:	90-106	99
	Group 3:	23- 89	74

Herd 87-25-0996

Relative Value	Group 1:	108-148	119
	Group 2:	88-107	97
	Group 3:	35- 87	78

Appendix G.
Release to Obtain Cooperator DHI Records

Date: ______________

I, ________________________, ________________________
(name) (title)

representing ____ give
(farm name)

Laura Mitchell and Dr. Robert C. Lamb permission to use my DHI records. I understand that they will be used as part of their study, "The Effects of GnRH and Milk Production on Pregnancy Rate in Repeat Breeder Cows." My DHI code is ________________.

____
(Signature)